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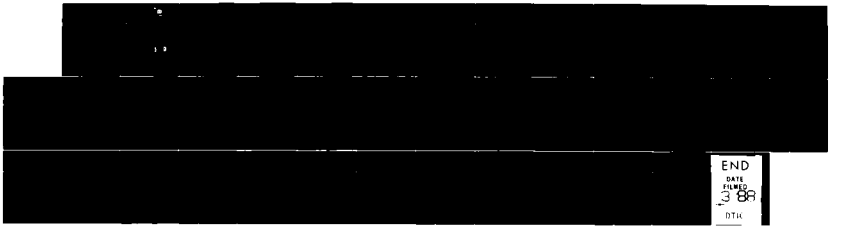

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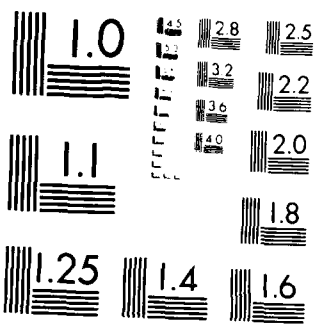

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US Army Corps
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Cold Regions Research &
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Analytical method for determining tetrazene in water

Marianne E. Walsh and Thomas F. Jenkins

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An ion-pairing RP-HPLC method was developed to determine tetrazene in water. The method uses an LC-18 column and a mobile phase of 2/3 v/v methanol-water modified by 0.01 molar 1-decanesulfonic acid sodium salt. The mobile phase pH was adjusted to 3 with glacial acetic acid. The modified mobile phase was optimal for separating of tetrazene from potential interferences by other explosive compounds such as HMX and RDX and for allowing elution of TNT within a 15-minute run time. The retention time for tetrazene was 2.8 minutes. The UV detector was set at 280 nm. A linear model with zero intercept was found to adequately describe the calibration data. The concentration range tested was 6.2-1238 µg/L. A spike recovery test on each of four days gave an average recovery of 103%. A reporting limit of 7.25 µg/L was estimated. The relative standard deviation was approximately 2% over the range tested. Tetrazene was found to be unstable in an aqueous medium at room temperature. Concentrations decreased by 96-100% over 24 hours. Chilled solutions were less prone to degradation than room temperature solutions, and heated solutions (50°C) degraded completely within two hours.

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PREFACE

This report was prepared by Marianne E. Walsh, Physical Scientist, Applied Research Branch, and Thomas F. Jenkins, Research Chemist, Geochemical Sciences Branch, U.S. Army Cold Regions Research and Engineering Laboratory. Funding for this research was provided by the U.S. Army Toxic and Hazardous Materials Agency, Aberdeen Proving Ground, MD (R-90 Multi-Analytical Services), Martin H. Stutz, Project Monitor.

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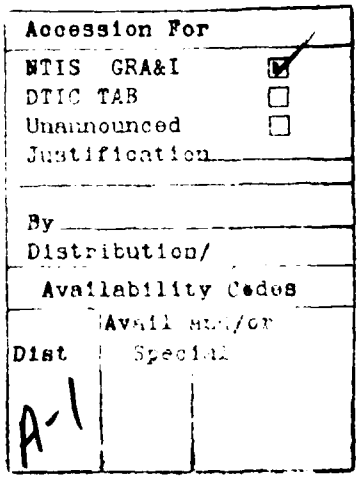

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ABBREVIATIONS

HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
RP-HPLC	Reverse-Phase, High-Performance Liquid Chromatography
SARM	Standard Analytical Reference Material
TNT	2,4,6-trinitrotoluene
USATHAMA	U.S. Army Toxic and Hazardous Materials Agency
UV	Ultraviolet

ANALYTICAL METHOD FOR DETERMINING TETRAZENE IN WATER

Marianne E. Walsh and Thomas F. Jenkins

INTRODUCTION

Tetrazene is an initiating explosive used as a component of primers and priming compositions. There has been no analytical protocol for determining tetrazene in water at levels of less than 500 μg/L. Twin City Army Depot in New Brighton, Minnesota, is required by the Environmental Protection Agency to monitor groundwater for tetrazene at the 10-μg/L level. USATHAMA asked this laboratory to develop a method for determining tetrazene in water with a reporting limit at or below 10 μg/L.

Literature Review

Quantitative methods for determining tetrazene in primer mixes described in the literature include polarographic, spectrophotometric and thermoanalytical protocols. Only one technique has been developed for determining tetrazene in aqueous media such as groundwater.

Wild (1957,1963) described a polarographic method for determining tetrazene in a single percussion cap. Interfering primer components were extracted with acetone and 0.01-molar hydrochloric acid, then the tetrazene was dissolved in 2.6-molar hydrochloric acid. This hydrochloric acid medium, modified with sodium tartrate, gave a well-defined reduction wave at the dropping mercury electrode. The height of the reduction wave was proportional to the concentration of tetrazene over the range of 26,320-191,760 μg/L.

Another polarographic method was developed by Black (1974), who dissolved tetrazene in cold 16% nitric acid. The supporting electrolyte was potassium nitrate, and the maximum suppressor was an aqueous solution of methylene blue. Diffusion current was linearly related to tetrazene concentration from 70 μg to 2.5 mg, or 2.8 to 100 mg/L.

Semel (1980) utilized differential pulse polarography for the analysis of NOL-130 primer mix. Hydrochloric acid was used to dissolve the entire sample, and 4-molar lithium chloride was the supporting electrolyte in tetrazene determinations. This method was used to quantify each component in 100 mg of NOL-130 primer mix, of which 5 mg should be tetrazene. Using the reagent quantities described in the method, this converts to a solution concentration of 20 mg/L.

Hetman (1964) used a potentiometric technique to titrate 154-218 mg of tetrazene in a nonaqueous formic-acetic acid solution using a perchloric-acetic acid solution as the titrant. For the reagent quantities described in this method, the solution concentrations were 3850-5450 mg/L.

Krien (1979) used the heat of decomposition to determine tetrazene in primer mixtures by differential scanning calorimetry. Quantitative determination of 0.1-1.5 mg of tetrazene was achieved using 2-5 mg of primer sample.

Tetrazene can also be determined spectrophotometrically. Norwitz and Keliher (1979) reacted tetrazene in primer mixes with resorcinol and measured the intensity of the yellow color of the diazo-dye formed. Synthetic mixes were prepared to simulate lead styphnate primers used in small arms. These mixtures ordinarily contain lead styphnate (35-40%), barium nitrate (35-45%), tetrazene (2-5%) and small percentages of anitmony trisulphide, nitrocellulose, PETN, powdered aluminum and calcium silicide. The primer samples were sequentially extracted, first with ammonium acetate solution to remove lead styphnate and barium nitrate, then with acetone to remove nitrocellulose and PETN. The remaining material was boiled with resorcinol reagent, and the absorbance was measured at 400 nm. In 250 mg of primer mix, between 6 and 13 mg of tetrazene was determined. Tummavuori and Surma-aho (1981) also extracted primer samples with ammonium acetate and acetone to remove components other than tetrazene. After being washed with water, the remaining sample was dissolved in formic acid, and the absorbance was measured at 278 nm. Concentrations of tetrazene were in the range of 2-5 mg/L.

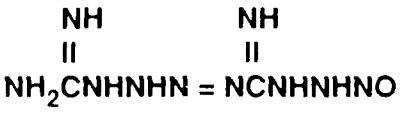
There are two USATHAMA-certified methods for determining tetrazene. Method 3J is a quantitative procedure for determining tetrazene in water in the concentration range of 500-10,000 μg/L. A spectrometer with a tungsten lamp and a wavelength set at 530 nm is used to detect color development

when a solution of sodium hydroxide, sodium nitroprusside and potassium ferricyanide is mixed with water contaminated with tetrazene. A detection limit of 500 $\mu g/L$ is estimated using the method of Hubaux and Vos (1970). No information is available, however, to assess its susceptibility to interferences. Method 3A is a qualitative test for tetrazene on surfaces. A color-developing reagent similar to the solution described in Method 3J is sprayed onto the surface to be tested. A positive test is indicated by the development of a red-orange color against a yellow background. A detection limit of 0.4 $\mu g/cm^2$ is claimed.

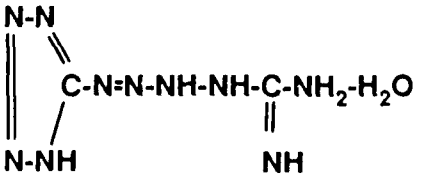
No chromatographic methods for determining tetrazene were found. Analysis by gas chromatography is prohibited by the thermal instability of tetrazene, and analysis by liquid chromatography is complicated by the limited solubility of tetrazene in water or common organic solvents.

Chemistry of Tetrazene

In 1910 Hofman and coworkers (Patinkin et al. 1955) treated aminoguanidium nitrate with sodium nitrite in neutral solution and isolated a white crystalline solid later named tetrazene [CAS REG No 31330-63-9, tetrazene-1-carboxamidine-4-(1H-tetrazol-5-yl) monohydrate]. Table 1 lists the physical constants of tetrazene. The structure of the compound was originally considered to be



until 1954, when Patinkin et al. (1955) proposed the following structure based on the results of degradative studies:



Two characteristics of tetrazene make analysis by chromatographic methods difficult: low solubility in water and most organic solvents, and poor thermal stability.

Table 1. Physical constants of tetrazene.

Empirical formula	$C_2H_8N_{10}O$*
Molecular weight	188.2*
Crystal density (g/cm^3)	1.7*
Energy of formation (kJ/kg)	+1130
Enthalpy of formation (kJ/kg)	+1005
Melting point (°C)	140-160 (explodes)*
Solubility (mg/L)	
Water	4.5†
Methanol	240†
Acetone	0.5†
Tetrahydrofuran	2†
Acetone	<d†

* Meyer (1981).
† Estimated by this laboratory.

Tetrazene dissolves readily in formic acid (Hetman 1964, Tummavuori and Surma-aho 1981), concentrated hydrochloric acid (Wild 1957, 1963), 4-N sulphuric acid (Traas and Ligtenberg 1962) and cold 16% nitric acid (Flack 1974). Preliminary tests conducted during this study indicated that tetrazene is practically insoluble in acetonitrile and tetrahydrofuran, and insoluble in acetone. Solubility in methanol was estimated by this laboratory to be 240 mg/L.

Tetrazene is thermally unstable, leading to a loss of activity as a sensitizer in primer caps (Elischer and Spear 1984, Whelan et al. 1984, Wild 1957). Tetrazene in aqueous solution decomposes completely upon boiling; for each mole of tetrazene hydrolyzed by boiling in water, 1.5-2.0 moles of nitrogen are produced along with ammonia, guanidine 1-H-tetrazole and 5-aminotetrazole (Ellis and Helton 1975). Solutions used as analytical standards are also known to decompose at room temperature (Traas and Ligtenberg 1962).

Objective

The objective of this research was to develop an analytical method for determining tetrazene in groundwater with a reporting limit of 10 $\mu g/L$ or lower. The method will be used to screen for contamination.

EXPERIMENTAL METHOD

Instrumentation

RP-HPLC determinations were conducted on a Perkin-Elmer series 3/LC65T High-Performance Liquid Chromatograph equipped with a variable-wavelength UV detector set at 280 nm and a Rheodyne 7125 sample loop injector. A 175-μL sample loop was overfilled by passing 500 μL of sample through the loop; the sample was then injected onto an analytical column. Columns tested included LC-8, LC-18, LC-CN, LC-DP and LC-Diol from Supelco, Inc. Numerous mobile phases were tested using various combinations of water and organic solvents such as methanol and acetonitrile. For the instrument calibration and the spike recovery study, an LC-18 column was eluted with 1.5 mL/min of a solvent consisting of 2/3 v/v methanol-water and 1-decanesulfonic acid sodium salt at a 0.01-molar concentration. The pH of this mobile phase was adjusted to 3 by adding 4 mL of glacial acetic acid to each liter of eluent. The mobile phase was chosen to minimize interferences from peaks observed in natural waters and to elute potential co-contaminants in a reasonable period of time.

Chemicals

Analytical standards for tetrazene were prepared from Standard Analytical Reference Materials (SARM) obtained from the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA), Aberdeen Proving Ground, Maryland. Standards were dried to a constant weight in a vacuum desiccator over dry calcium chloride in the dark. The methanol used to prepare the tetrazene standards, and the mobile phase for HPLC analysis was either Mallinckrodt ChromAR HPLC or Baker HPLC grade. The ion-pairing reagent for HPLC was 1-decanesulfonic acid sodium salt, 98%, obtained from Aldrich. The glacial acetic acid was Mallinckrodt (99.5%). Water used for spike recovery, dilution of standards, and preparation of the mobile phase was purified by a MilliQ Type I Reagent Grade Water System (Millipore). The mobile phase was vacuum filtered through a Whatman GF-F microfiber filter to remove particulates and degas the eluent.

Optimum Detector Wavelength

The optimum wavelength setting on the variable UV detector was determined by repeated analysis of the same tetrazene sample at settings ranging from 240 to 305 nm in increments of 5 nm.

Stability Study

In the initial phase of this study, we observed that solutions of tetrazene in water or methanol, or both, were unstable over time. Before quantitative analyses could be performed, the calibration standards and aqueous samples had to be stabilized. A study was conducted to see if low-temperature storage would slow degradation.

Two test solutions of tetrazene were prepared by adding water or methanol to vials containing dried SARM. The vials were capped, shaken and allowed to stand several weeks. Undissolved tetrazene remained on the bottom of each vial. The aqueous tetrazene solution was diluted 1/99 v/v with water, vacuum-filtered through a 0.45-μm Nylon-66 Supelco filter membrane and chilled to 4°C in an ice bath. The methanol-tetrazene solution was diluted 0.5/99.5 v/v with methanol, filtered and chilled in the same manner as the aqueous solution. Samples of each of these solutions were analyzed over four days. On two of the days, subsamples of each solution were taken and allowed to warm to room temperature. These solutions were analyzed along with the chilled solutions over a 24-hour period.

Calibration Standards

An analytical stock standard of tetrazene was prepared by accurately weighing approximately 10 mg of dried SARM into a tared glass vial. Methanol was added to the vial, and the methanol-tetrazene suspension was transferred through a funnel into a 100-mL volumetric flask with a ground glass stopper. The vial was rinsed five times with methanol, and the rinse was added to the flask prior to its being filled to volume. A stir bar was added to the flask, and the flask closure was wrapped in parafilm. Then the flask was placed in an ice bath on top of a stirring plate for 45 minutes, after which crystals of tetrazene were no longer visible.

To test the linearity of the detector response, the stock solution was allowed to warm to room temperature, and a series of intermediate standards were prepared by pipetting the volumes shown in Table 2 into individual volumetric flasks. The stock solution and the diluted standards were maintained at 4°C throughout the analysis. For each working standard, 20.0 mL of water was added to a glass vial, and the vial was placed in an ice bath. Then 250 μL of standard was added to the chilled water. The vial was capped and shaken, and then a portion of the diluted standard was analyzed. Duplicate analyses were performed for each concentration level.

Table 2. Calibration standards for tetrazene.

Aliquot of stock (mL)	Capacity of volumetric flask (mL)	Solution concentration (µg/L)	Concentration* in water (µg/L)
0.5	100	495	6.11
1	100	990	12.2
2	100	1,980	24.4
5	100	4,950	61.1
10	100	9,900	122.2
20	100	19,800	244.4
25	50	49,500	611.1
Stock	no dilution	99,000	1222.2

* Concentrations correspond to dilution of 250 µL of standard with 20.0 mL of water.

Spike Recovery Study

Reporting limits were obtained using the Hubaux and Vos (1970) method outlined in the USATHAMA Installation Restoration Program Quality Assurance Program for Class 1 certification. Samples of reagent-grade water were spiked and analyzed on four separate days. The spiking solution stock for the recovery study was prepared in a manner similar to that described for the calibration standard stock, except that 5.5 mg of SARM material was used. A series of spiking solutions was prepared by the dilutions shown in Table 3.

Table 3. Spiking solutions for spike recovery test.

Aliquot of stock (mL)	Capacity of volumetric flask (mL)	Solution concentration (µg/L)	Concentration* in water (µg/L)
0.5	50	580	7.16
1	50	1,160	14.3
2	50	2,320	28.6
5	50	5,800	71.6
5	25	11,600	143
4	10	23,200	286
Stock	no dilution	58,000	716

* Assuming 250 µL of spike solution added to 20.0 mL of water.

Samples were prepared by pipetting 20.0 mL of reagent-grade water into a series of glass vials. The vials were placed in an ice bath. Each vial of chilled water was spiked with 250 μL of one of the spiking solutions. Prior to analysis, each water sample was filtered through a 0.45-μm Millex HV disposable filter unit using a 20-mL disposable BD syringe. The first 5 mL of filtrate was discarded, and the remaining 15 mL was saved for analysis.

RESULTS AND DISCUSSION

Column and Eluent Selection

On each of the analytical columns tested, tetrazene eluted rapidly (i.e., the retention time was less than 2 minutes) when the mobile phase consisted of a combination of water and an organic solvent such as methanol or acetonitrile. Such a short retention time means that tetrazene is eluting near the composite peak of all the unretained components, thus increasing the possibility of interference in real environmental samples. An LC-18 column eluted with 100% water produced a tetrazene retention time of 6.3 minutes. However, HMX and RDX had retention times of 31.5 and 47 minutes, respectively. The very long run times for samples where these components were present would be unacceptable. Ideally the column-eluent combination should elute tetrazene without interference and elute other potential contaminants within a reasonable run time. While gradient elution could minimize this problem, the equilibration time between runs would significantly decrease the daily sample output, and not all HPLC systems are equipped to do gradient elution.

While we were unable to locate the pK_b of tetrazene, its structure suggests it is a weak base, with some portion of the molecule existing in the ionized form at neutral or acidic pH. This supposition is consistent with tetrazene's chromatographic behavior on reverse-phase columns in which it is generally unretained when eluents contain a significant organic component. We therefore tried ion-pairing chromatography. The pH of the mobile phase was buffered in a range where tetrazene exists predominantly as a substituted ammonium cation.

Ion-pairing chromatography uses a reverse-phase column and an eluent modified by the addition of an ion-pairing reagent. Molecules of the ion-

pairing reagent contain a charged end of opposite charge to the analyte of interest) and a long hydrocarbon chain that can interact hydrophobically with the stationary phase. For ammonium compounds a sodium salt of a long-chain alkylsulfonic acid is often used as the ion-pairing reagent, forming stable ion pairs that can interact as a unit with the hydrocarbon-like surface of the reversed phase. This interaction causes the analyte to be retained to a greater extent than it would otherwise. It also allows the use of stronger eluents (higher percentages of the organic component) so that other non-ionic components can be eluted at reasonable retention times. The ion-pairing reagent selected was 1-decanesulfonic acid sodium salt at an eluent concentration of 0.01 molar. The pH of the mobile phase was adjusted to about 3 with glacial acetic acid to ensure complete ionization of tetrazene. For this analysis the required amount of glacial acetic acid was 4 mL per liter of eluent. The retention time for tetrazene was 2.8 minutes using an eluent composed of 2/3 v/v methanol-water, 0.01-molar ion-pairing reagent at pH 3. Retention times for HMX, RDX and TNT were 3.6, 6.0 and 12.9 minutes, respectively. Figure 1 shows a typical chromatogram for these analytical conditions.

The tetrazene retention time can be adjusted to suit the needs of a particular analytical situation. Table A1 is a list of the various eluents tested during the method development and the corresponding retention times

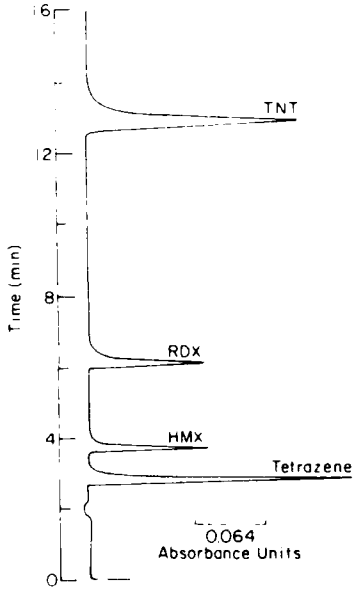


Figure 1. Typical chromatogram showing separation of tetrazene from other explosives.

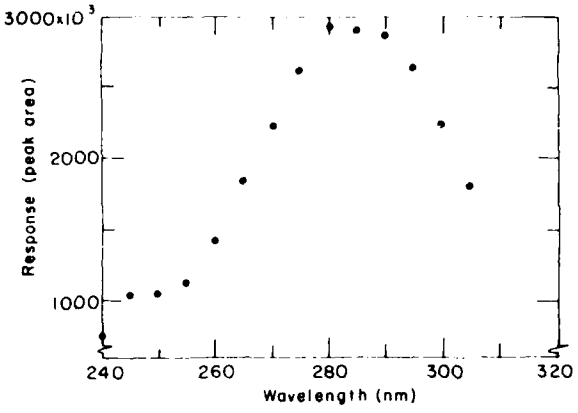


Figure 2. Determination of optimum wavelength for detection of tetrazene.

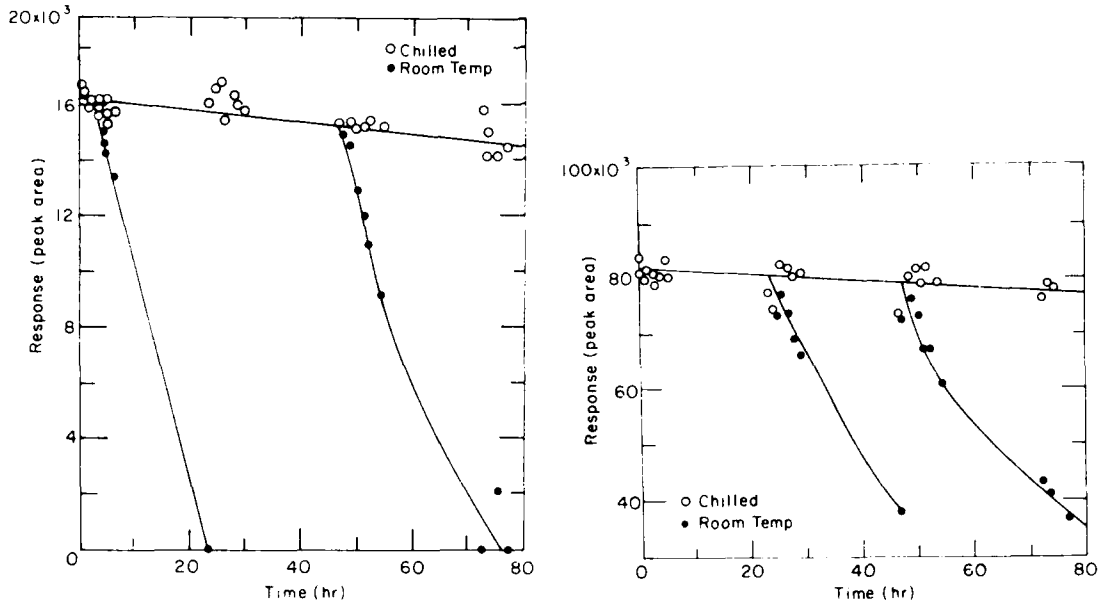
of tetrazene. Clearly the retention time is influenced not only by the ratio of methanol to water but also by the percent by volume of acetic acid; the molar concentration of the ion-pairing reagent appears to be less important.

Optimum Detector Wavelength

The response at various detector wavelengths is presented in Figure 2 (Table A2). The maximum response is in the region of 280-285 nm. Since 280-nm fixed-wavelength detectors are commercially available, 280 nm was selected for this analysis.

Stability Study

Stable calibration standards are necessary for a quantitative determination of tetrazene in water. During the development of this chromatographic method, we observed that the detector response for injected tetrazene standards decreased with time. Responses for aqueous samples prepared in the morning and maintained at room temperature gave noticeably lower responses by the end of the day. The pH of aqueous samples was adjusted in an attempt to stabilize the solutions. Samples adjusted to pH values of 2.5-3.7 and 9.6-11.3 degraded, as did the untreated samples. Samples at the pH extremes of 2.5 and 11.3 degraded the fastest. Since tetrazene degrades at temperatures greater than 50°C, the stability of chilled solutions was tested. Figure 3 shows the detector response of aqueous-tetrazene and methanol-tetrazene solutions at room temperature and at 0°C over



a. Tetrazene in water.

b. Tetrazene in methanol.

Figure 3. Effect of temperature on the stability of tetrazene solutions.

four days. Integrator peak areas are presented in Table A3. Clearly degradation was slowed by maintaining the solutions at low temperature. For example, the detector response during 24 hours decreased by only 3% for the chilled aqueous samples as opposed to 96-100% for the room temperature standards. The degradation of tetrazene was slower in methanol than in the aqueous solutions. The response declined by 1% and 27% for the chilled and room temperature methanol samples, respectively, in 24 hours.

Instrument Calibration

To determine if the detector response was a linear function of analyte concentration, the calibration data were subjected to a regression analysis for a non-zero-intercept linear model ($y = a + bx$) and a zero-intercept model ($y = bx$). The regression coefficients a and b were estimated using the method of least squares (Tables A4 and A5).

The fitted equations for both models were subjected to the lack-of-fit (LOF) test. A linear model was found to be acceptable at the 95% confidence level. The intercept was then tested to determine if it was significantly different from zero. The F-ratio was calculated by dividing

the difference between the residual sum of squares for the non-zero- and zero-intercept models by the residual mean square for the model with non-zero intercept. Since the calculated F-ratio was less than the critical value at the 95% confidence level, the zero-intercept linear model was accepted. Thus, daily calibration can be obtained using a zero-intercept model.

Spike Recovery Study

A spike recovery study was conducted to allow estimation of the method reporting limit. Spike solutions were prepared such that the spiked water samples would have analyte concentrations ranging from 0.5 to 50 times the target reporting limit. The results are presented in Tables A6-A8.

The certified reporting limit was calculated using the method of Hubaux and Vos (1970). First the mean and variance at each target level were calculated, and the variances were compared using Bartlett's test (Table A9). The range of homogeneous variance was limited to the concentration range of 7.25 to 29 μg/L. Therefore, the data set used to calculate the reporting limit included the blank and the three lowest target concentrations.

The data were analyzed statistically using software provided by USATHAMA. The entire data set (i.e. the blank and 0.5-50 times the target reporting limit) was entered into the computer. The data from each of the four days were pooled and tested for lack of fit. The method reporting limit was then obtained from the X value corresponding to the point on the lower confidence limit curve where the Y value matches the value of Y on the upper confidence limit curve at X=0 (Fig. 4). The data set was sequentially truncated, starting with the highest concentration, until the range of homogeneous variance was reached. A method reporting limit of 3.04 μg/L was calculated. The analytical precision as determined from the percent relative standard deviation was roughly 2% over the concentration range of 14.5-725 μg/L and 15% for the lowest spike level.

As dictated by the USATHAMA protocol, the certified reporting limit must be equal to or greater than the lowest tested concentration. Otherwise the lowest tested concentration is the minimum value that can be reported as the certified reporting limit. In this case the lowest tested

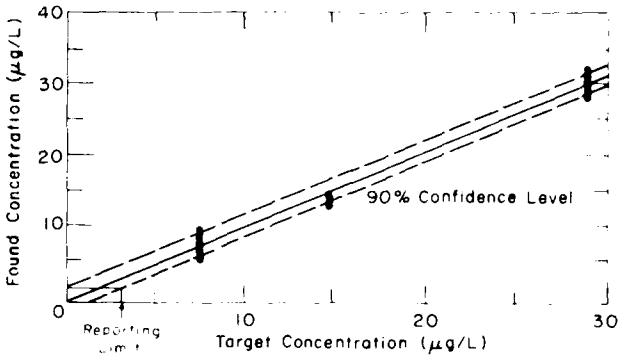


Figure 4. Reporting limit determination for tetrazene.

concentration was 7.25 μg/L, and the calculated reporting limit was 3.04 μg/L. Therefore, the certified reporting limit is reported as 7.25 μg/L.

SUMMARY AND CONCLUSIONS

A method was developed for determining tetrazene in water. The method involves: 1) maintenance of samples at 4°C, 2) filtration of cold aqueous samples through a 0.45-μm membrane, and 3) analysis by an ion-pairing HPLC technique. An LC-18 column is eluted with a methanol-water 2/3 v/v eluent modified with 1-decanesulfonic acid sodium salt and glacial acetic acid. Tetrazene was detected with a variable-wavelength UV detector set at 280 nm. The tetrazene retention time using this method was 2.8 minutes. The eluent was chosen to allow elution of TNT, a possible contaminant, within 15 minutes. Other tested eluents resulted in a longer retention time for tetrazene. These eluents may be appropriate if TNT is not present in the sample.

The variances from each target level were compared using Bartlett's test. The range of homogeneous variance was limited to the concentration range of 7.25-29 μg/L. For data over this concentration range, the method reporting limit was estimated to be 3.04 μg/L. Since this value is less than the lowest concentration tested, 7.25 μg/L is the certified reporting limit.

LITERATURE CITED

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Appendix A: Data

Table A1. Eluents tested for tetrazene elution.

Ratio of $MeOH-H_2O$ (V/V)	Concentration of 1-decanesulfonic acid, sodium salt (M)	Concentration of acetic acid (% by volume)	Retention time (min)
1/1	0	0	2.2
1/1	0.01	0.8	2.2
2/3	0.005	0.15	2.5
2/3	0.005	0.5	2.8
2/3	0.005	0.7	2.8
2/3	0.0074	0.8	3.0
2/3	0.01	0.4	2.8
1/2	0.0067	0.1	2.8
1/2	0.0067	0.55	3.5
1/3	0	0.8	2.8
1/3	0.005	0.3	5.0
1/3	0.005	0.4	5.9
1/3	0.01	0.15	4.2
1/3	0.01	0.3	4.8
1/3	0.01	0.4	5.0
1/3	0.01	0.5	6.2
1/9	0.01	0.3	12.2

Table A2. Response of UV detector to tetrazene at various wavelengths.

λ(nm)	Response*
240	766,480
245	1,041,600
250	1,060,400
255	1,109,500
260	1,414,000
265	1,848,000
270	2,254,300
275	2,621,000
280	2,913,000
285	2,906,200
290	2,893,000
295	2,653,700
300	2,241,000
305	1,809,500

* Response in peak areas to 1,212.5 µg/L of tetrazene standard.

Table A3. Stability of tetrazene in chilled solutions.

Aqueous samples				Methanol samples			
Chilled		Room temp.		Chilled		Room temp.	
Time (hours)	Response (peak area)	Time (hours)	Response (peak area)	Time (hours)	Response (peak area)	Time (hours)	Response (peak area)
0	16625	-	-	0	83276	-	-
0.13	16342	-	-	0.08	80762	-	-
1.65	15826	-	-	1.50	79038	-	-
1.88	15990	-	-	1.73	81062	-	-
2.87	16093	-	-	2.78	80884	-	-
2.97	15543	-	-	2.90	78837	-	-
3.97	15668	4.41	15011	3.93	80128	-	-
4.08	16178	4.51	14540	4.03	80195	-	-
4.77	16110	5.18	14217	4.70	80247	-	-
4.85	15681	5.28	13366	4.80	83109	-	-
23.15	15933	23.24	no peak	23.12	77166	-	-
24.80	16504	-	-	24.22	74658	24.65	73221
25.02	16617	-	-	25.68	82139	25.50	76859
25.87	15358	-	-	26.73	81434	26.85	73142
27.20	16128	-	-	27.82	80173	27.93	68512
27.57	15876	-	-	28.85	80863	28.93	66322
29.30	15624	-	-	46.95	73015	47.05	28358
47.15	15267	47.42	14880	48.52	80462	-	-
48.58	15237	48.68	14420	49.98	81222	47.20	72896
49.92	15018	50.00	12823	50.97	78819	48.60	75972
51.07	15112	51.15	11936	51.68	81470	50.07	72635
51.78	15204	51.90	10987	53.95	78828	51.05	66997
54.05	15136	54.16	9016	72.30	76292	51.77	66669
72.42	15767	72.50	no peak	73.43	78681	54.07	60651
73.33	14107	75.15	2172	76.93	78058	72.48	33308
73.57	14940	76.98	no peak			73.53	31341
75.05	14028	-	-			77.05	26518
76.85	14419	-	-				

Table A4. Instrument calibration results for tetrazene.

Standard concentration (µg/L)	Injection concentration (µg/L)*	Response (peak areas)	
		Replicate 1	Replicate 2
99,000	1222.22	3,127,700	3,028,000
49,500	611.11	1,505,000	1,471,000
19,800	244.44	600,130	594,840
9,900	122.22	322,860	300,870
4,950	61.11	157,840	154,310
1,980	24.44	63,207	63,732
990	12.22	33,673	32,342
495	6.11	16,558	20,050
0	0	0	0

* Concentration corresponds to dilution of 250 mL of standard with 20.0 mL of water.

Table A5. Lack-of-fit (LOF) and zero-intercept tests for calibration standards.

PRE-CERTIFICATION ANALYSIS — Report Date: 10/09/87
Page: 1

Method Name: HPLC — Laboratory: CB
Compound: TETRAE — Analysis Date: 05/04/87
Units of Measure: UGL — Matrix: WA

ANALYSIS OF RESIDUAL VARIATIONS

	--- Model with Intercept --- Y = (-2739.94948) + (2503.602890)X			- Model through the Origin - Y = (2500.359870)X		
	(SS)	(df)	(MS)	(SS)	(df)	(MS)
Residual:	10410103400	14	7435788814.3	10489277500	15	6992851666.7
Total Error:	5817168180	8	727146022.5	5817168180	8	727146022.5
Lack of Fit:	4592935220	6	765489203.3	4672109320	7	667444188.6

LOF F-Ratio(F): 1.052731060 — LOF F-Ratio(F): 0.917895674
Critical 95% F: 3.58 — Critical 95% F: 3.50

ZERO INTERCEPT HYPOTHESIS

Zero Intercept Accepted — Calculated F: 0.106477079 Critical 95% F: 4.60

****

TABLE OF DATA POINTS — Targets: 8 — Measures per Target: 2

	Target Value	Instrument Values	
8:	6.1100000	16558	20050
7:	12.220000	33673	32342
6:	24.440000	63207	63732
5:	61.110000	157840	154310
4:	122.22000	322860	300870
3:	244.44000	600130	594840
2:	611.11000	1505000	1471000
1:	1222.2200	3127700	3028000

*** END OF PRE-CERTIFICATION DATA TABLE ***

Table A6. Spike recovery study: HPLC analysis.

Sample	Target concentration (µg/L)	Response (peak area) Day 1	Day 2	Day 3	Day 4
Blank	0	0	0	0	0
		0	0	0	0
0.5x	7.25	19580	17035	16557	21076
		17274	21629	24789	16613
1x	14.5	32305	31715	36929	35120
		32265	33497	35174	37792
2x	29	70247	70776	73661	75394
		66228	66037	74098	76262
5x	72.5	156880	173920	177900	175670
		162390	169900	178130	177720
10x	145	310180	332560	345960	350030
		317310	326500	351660	339960
20x	290	638670	668710	704520	700190
		636540	665750	709810	701660
50x	725	1627500	164200	1749200	1773800
		1560400	165900	1738100	1779100
Standard	1197.53	2708700	2814500	3040100	2964400
		2703600	2733500	2915400	2966400
		2659900	2760300	3008900	3027600
		2661200	2780900	3006100	2947700
		2577900	2716700	2986200	2987400
		2545600	2703000	2991800	2951500

Table A7. Spike recovery results.

Target conc. (µg/L)	Found concentration (µg/L)							
	Day 1		Day 2		Day 3		Day 4	
725	737.57	707.16	714.30	722.00	700.24	695.80	714.21	716.34
290	289.44	288.48	291.04	289.75	282.03	284.15	281.93	282.52
145	140.57	143.80	144.74	142.10	138.50	140.78	140.94	136.88
72.5	71.10	73.59	75.70	73.94	71.22	71.31	70.73	71.56
29	31.84	30.01	30.80	28.74	29.49	29.66	30.38	30.71
14.5	14.64	14.62	13.80	14.58	14.78	14.08	14.14	15.22
7.25	8.87	7.83	7.41	9.41	6.63	9.92	8.49	6.69
0	0	0	0	0	0	0	0	0

Table A8. Computer output from THAMA IRPQAP software including calculated reporting limit.

CERTIFICATION ANALYSIS — Report Date: 07/27/87

Method Name: RP-HPLC — Laboratory: CR
Compound: TETRAE — Analysis Date: 05/05/87
Units of Measure: UGL — Matrix: WA

TABLE OF RESULTS FOR TRUNCATED DATA SET

Target Concentration	Standard Deviation	Percent Inaccuracy	Percent Imprecision
7.2500000	1.2216024	12.500000	14.977501
14.500000	0.4520983	-0.120690	3.1216867
29	0.9485845	4.1422414	3.1408782
72.500000	1.7905461	-0.146552	2.4733435
145	2.5770107	-2.731897	1.8271650
290	3.8745018	-1.321552	1.3539280
725	13.039061	-1.592759	1.8276004

CERTIFICATION ANALYSIS — Report Date: 07/27/87

Method Name: RP-HPLC — Laboratory: CR
Compound: TETRAE — Analysis Date: 05/05/87
Units of Measure: UGL — Matrix: WA

TABLE OF DATA POINTS

Target Concentration	Found Concentration
0	0
	0
	0
	0
	0
	0
	0
	0
7.2500000	8.8700000
	7.8300000
	7.4100000
	9.4100000
	6.6300000
	9.9200000
	8.4900000
	6.6900000

Table A8 (cont'd). Computer output from THAMA IRPQAP software including calculated reporting limit.

CERTIFICATION ANALYSIS **Report Date: 07/27/87**

Method Name: RP-HPLC **Laboratory:** CR
Compound: TETRAH **Analysis Date:** 05/05/87
Units of Measure: UGL **Matrix:** WA

TABLE OF DATA POINTS

Target Concentration	Found Concentration
14.500000	14.640000
	14.620000
	13.800000
	14.580000
	14.780000
	14.080000
	14.140000
	15.220000
29	31.840000
	30.010000
	30.800000
	28.740000
	29.490000
	29.660000
	30.360000
	30.710000
72.500000	71.100000
	73.590000
	75.700000
	73.940000
	71.220000
	71.310000
	70.730000
	71.560000

Table A8 (cont'd).

CERTIFICATION ANALYSIS

Report Date: 07/27/87

Method Name: RP-HPLC
Compound: TETRAE
Units of Measure: UGL

Laboratory: CE
Analysis Date: 05/05/87
Matrix: WA

TABLE OF DATA POINTS

Target Concentration	Found Concentration
145	140.57000
	143.80000
	144.74000
	142.10000
	138.50000
	140.78000
	140.94000
	136.88000
290	289.44000
	288.48000
	291.04000
	289.75000
	282.03000
	284.15000
	281.93000
	282.52000
725	737.57000
	707.16000
	714.30000
	722
	700.24000
	595.80000
	714.21000
	716.34000

Table A8 (cont'd). Computer output from THAMA IRPQAP software including calculated reporting limit.

CERTIFICATION ANALYSIS — Report Date: 07/27/87

Method Name: RP-HPLC — Laboratory: CR
Compound: TETRAE — Analysis Date: 05/05/87
Units of Measure: UGL — Matrix: WA

-- REGRESSION EQUATION --
Y = 0.9832122X + 0.5231765

-- UPPER REPORTING LIMIT --
725

-- SLOPE --
0.9832122

SUMMARY TRUNCATION TABLE

Target Concentrations Used	Slope	% Change from Total Data Set	% Change from Previous Data Set
Entire data set	0.9832122	0	0
minus 1 highest	0.9822442	0.0984532	0.0984532
minus 2 highest	0.9706004	1.2827208	1.1854347
minus 3 highest	0.9953492	1.2344199	2.5498482
minus 4 highest	1.0318227	4.9440430	3.6643891

Target Concentrations Used	Certified Reporting Limit	Upper Reporting Limit
Entire data set	16.538097	725
Minus 1 highest	7.3798416	725
Minus 2 highest	5.6703748	725
Minus 3 highest	4.0547933	725
Minus 4 highest	3.0400479	725

Table A8 (cont'd).

CERTIFICATION ANALYSIS — Report Date: 07/27/87

Method Name: RP-HPLC — Laboratory: CR
Compound: TETRAE — Analysis Date: 05/05/87
Units of Measure: UGL — Matrix: WA

ANALYSIS OF RESIDUAL VARIATIONS

	--- Model with Intercept --- Y = (0.641667920) + (0.982973563)X			- Model through the Origin - Y = (0.984265993)X		
	(SS)	(df)	(MS)	(SS)	(df)	(MS)
Residual:	1433.986510	54	26.55530574	1448.530110	55	26.33691109
Total Error:	1382.307000	49	28.21034694	1382.307000	49	28.21034694
Lack of Fit:	51.67951000	5	10.33590200	66.22311000	6	11.03718500

LOF F-Ratio(F): 0.366386915 — Critical 95% F: 2.45
LOF F-Ratio(F): 0.391245986 — Critical 95% F: 2.34

ZERO INTERCEPT HYPOTHESIS

Zero Intercept Accepted — Calculated F: 0.547672098 — Critical 95% F: 4.08

****

TABLE OF DATA POINTS — Targets: 7 — Measures per Target: 8

	Target Value	Found Concentration				
1:	7.2500000	8.8700000	7.8300000	7.4100000	9.4100000	6.6300000
		9.9200000	8.4900000	6.6900000		
2:	14.500000	14.640000	14.620000	13.800000	14.580000	14.780000
		14.080000	14.140000	15.220000		
3:	29	31.840000	30.010000	30.800000	28.740000	29.490000
		29.660000	30.360000	30.710000		
4:	72.500000	71.100000	73.590000	75.700000	73.940000	71.220000
		71.310000	70.730000	71.560000		
5:	145	140.57000	143.80000	144.74000	142.10000	138.50000
		140.78000	140.94000	136.88000		
6:	290	289.44000	288.48000	291.04000	289.75000	282.03000
		284.15000	281.93000	282.52000		

Table A8 (cont'd). Computer output from THAMA IRPQAP software including calculated reporting limit

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CERTIFICATION ANALYSIS                                  Report Date: 07/27/87
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Method Name:      RP-HPLC                    Laboratory:    CR
Compound:         TETRAE                     Analysis Date: 05/05/87
Units of Measure: UGL                        Matrix:        WA

TABLE OF DATA POINTS              Targets: 7     Measures per Target: 8

     Target Value  Found Concentration

7:     725         737.57000  707.18000  714.30000  722        700.24000
                   695.80000  714.21000  716.34000

*** END OF CERTIFICATION LACK OF FIT DATA TABLE ***
```

Table A9. Means and variances of found concentrations at each target level.

Target concentraton (µg/L)	Found concentration (µg/L) Mean	Variance	Bartlett's test (X^2)
0	0	0*	
7.25	8.156	1.49	0
14.5	14.48	0.204	5.94
29.0	30.20	0.900	5.89
72.5	72.39	3.20	10.98**
145.0	140.99	7.09	19.58**
290.0	286.17	15.01	31.32**
725.0	713.45	170.11	91.09**

* Results for blank not used in Bartlett's test.

** X^2 value above the critical value at 95% confidence level.

Appendix B: Method in USATHAMA Format

REVERSE-PHASE HPLC METHOD FOR THE DETERMINATION OF TETRAZENE IN WATER

I. SUMMARY

A. ANALYTES. The compound tetrazene can be determined using this method.

B. MATRIX. This method is suitable for determination of tetrazene in water.

C. GENERAL METHOD. The method involves filtration of water sample followed by determination using ion-pairing reverse-phase HPLC - UV 280 nm.

II. Application

A. TESTED CONCENTRATION RANGE

Linearity tests were conducted using peak area measurements. For a 175-μL injection volumn, this method was found to be linear over the concentration range of 6.11-1222.2 μg/L.

B. SENSITIVITY. The response of the UV detector at 280 nm for tetrazene was estimated at 0.45 absorbance units/mg/L using the conditions described below and a 175-μL injection volume.

C. REPORTING LIMIT. The reporting limit as determined over four days using the method of Hubaux and Vos was 7.25 μg/L using a 175-μL injection volume.

D. INTERFERENCES. No interferences were found. However, tetrazene elutes early, and if a computing integrator is used for peak quantitation, the baseline setting may have to be customized to exclude baseline aberrations. While these aberrations are insignificant when high concentrations of tetrazene are determined, they can cause large errors when low concentrations are determined. A blank run will help determine where the true baseline should be set.

E. ANALYSIS RATE. Approximately 40 samples can be analyzed in a day, provided the samples are not contaminated with late-eluting compounds such as TNT.

F. SAFETY INFORMATION. Tetrazene is extremely explosive in the dry state. Only small portions of the SARM material should be dried to prepare

analytical standards. Methanol is a flammable organic solvent, and established safety precautions should be used.

III. APPARATUS AND CHEMICALS

A. GLASSWARE/HARDWARE

1. Injection syringe - Hamilton, liquid syringe, 500-μL
2. Filters - 0.5-μm Millex-HV, disposable
3. Pipettes - 4.0-mL and 6.0-mL volumetric, glass
4. Scintillation vials - 20-mL, glass
5. Disposable syringes - Plastipak, 10-mL
6. Analytical Balance - ±0.1 mg

B. INSTRUMENTATION

1. HPLC - Perkin Elmer Series 3 (or equivalent) equipped with a 175-μL loop injector and a 280-nm UV detector.
2. Strip chart recorder.
3. Digital integrator - HP-3390 (or equivalent).
4. LC-18 (Supelco) RP-HPLC column, 25-cm x 4.6-mm (5μm)

C. ANALYTE

tetrazene
boiling point - NA
melting point - 140-160°C
solubility in water at 22°C is 4.5 mg/L
CAS REG No 31330-63-9

D. REAGENTS AND SARMS

1. Tetrazene - SARM quality
2. Methanol - HPLC grade
3. Water - Reagent grade
4. 1-Decanesulfonic acid, sodium salt - HPLC grade
5. Glacial Acetic Acid - reagent grade

IV. CALIBRATION

A. INITIAL CALIBRATION

1. Preparation of Standards. SARM is dried to constant weight in a vacuum desiccator in the dark. About 10 mg are weighed into a 100-mL volumetric flask and diluted to volume with methanol. The flask is inverted several times until tetrazene is dissolved. The stock solution is stored in the freezer at -10°C in the dark. The stock solution concentration is about 100 mg/L and is usable for one week from date of preparation.

A series of intermediate standards are prepared by diluting the stock. Intermediate calibration standards containing 0, 0.5, 1, 2, 5, 10 and 20 mg/L are prepared by placing 0, 0.5, 1, 2, 5, 10 and 20 mL of stock in a series of 100-mL volumetric flasks and filling to volume with methanol. An intermediate standard containing 50 mg/L is prepared by placing 25.0 mL in a 50-mL volumetric flask and filling to volume with methanol.

Injection standards are prepared by diluting 250 µL of each of the intermediate standards in 20.0 mL of water. The resulting concentrations will be 0, 6.2, 12.4, 24.8, 62.0, 124, 240, 620 and 1240 µg/L.

All solutions should be either refrigerated or kept in an ice bath following dilution.

2. Instrument Calibration. Duplicate injections of each standard over the concentration range of interest are sequentially analyzed in random order. Peak areas or peak heights are obtained. The retention time is 2.8 min.

3. Analysis of Calibration Data. The acceptability of a linear model with zero intercept is assessed using the protocol specified in USATHAMA QA (2nd Edition, March 1987). Experience indicates a linear model with zero intercept is proven to be appropriate; thus the slope of the best-fit regression line is then equivalent to a response factor. This response factor can be compared with values obtained from replicate analyses of a single calibration standard each day.

B. DAILY CALIBRATION. The stock standard can be used for daily calibration. A 250-µL aliquot of this stock is added to 20.0 mL of water in a scintillation vial. This standard is analyzed in triplicate at the beginning of the day, singly after each five samples and singly after the last sample of the day. The standard is maintained at 4°C throughout the

analyses. A response factor is obtained from the mean peak area or peak height obtained over the course of the day and compared with the response factor obtained for the initial calibration. These values must agree within ±10%, or a new initial calibration must be obtained.

V. CERTIFICATION TESTING

A. PREPARATION OF SPIKING SOLUTIONS. An analyte spiking solution is prepared in a manner identical to that described for the calibration stock except that 11 mg of SARM material is weighed into a 200-mL volumetric flask. A series of spiking standards (0, 0.28, 0.55, 1.1, 2.8, 5.5 and 11 mg/L) are prepared by placing 0, 0.5, 1.0, 2.0, 5.0, 10 and 20 mL of the stock in a series of 100-mL volumetric flasks and diluting to volume with methanol. The spike solution containing 28 mg/L is prepared by placing 25 mL into a 50-mL volumetric flasks and diluting to volume with methanol. The stock serves as a 55-mg/L spiking solution.

B. PREPARATION OF CONTROL SPIKES. Spiked water samples containing 0, 3.4, 6.8, 14, 34, 68, 140, 340 and 680 μg/L are prepared by injecting 250 μL of each spiking standard, including the stock, into 20.0 mL of water. Duplicate spiked water samples are prepared.

C. ANALYSIS OF WATER SPIKES. Water spikes are processed and analyzed as described below for real samples.

VI. SAMPLE HANDLING AND STORAGE

A. SAMPLING PROCEDURE. Representative subsamples are taken for analysis.

B. CONTAINERS. All containers used to store water samples should be cleaned according to procedures specified in the USATHAMA QA Manual and rinsed with water.

C. STORAGE. All water samples must be stored at 4°C before and throughout the analysis.

D. HOLDING TIME LIMITS. Samples should be processed as soon as possible after receipt, preferably within a day.

VII. PROCEDURE

A. FILTRATION. A 10-mL portion of each water sample is placed in a Plastipak syringe and filtered through a 0.5-μm Millex-HV filter unit. The first 5 mL of filtrate are discarded, and the remainder is retained for analysis.

B. DETERMINATION. Determination of analyte concentration in the filtered water samples is obtained by ion-pairing RP-HPLC on a 280-nm UV detector. The eluent is prepared by adding to a solution of 2/3 v/v methanol-water enough 1-decanesulfonic acid, sodium salt to obtain a 0.01-molar concentration level and adjusting the pH to about 3 with glacial acetic acid. For 1 L of eluent, 2.44 g of 1-decanesulfonic acid, sodium salt are dissolved in 400/600 v/v methanol-water and 2.0 mL of glacial acetic acid added to the mixture. A 175-μL loop is overfilled by injecting 500 μL of sample through the loop and onto an LC-18 column eluted at 1.5 mL/min. The retention time for tetrazene is 2.8 min, and a capacity factor based on an unretained peak for nitrate is 0.795. A chromatogram obtained for tetrazene and potential interferences is shown in Figure 1.

VIII. CALCULATIONS

A. RESPONSE FACTOR. Since a linear calibration curve with zero intercept is to be expected, calculation of results on a daily basis is obtained using a response factor. The mean response ($\bar{R}$) for tetrazene is obtained in either peak area or peak height units. The response factor is obtained by dividing the mean response by the known solution concentration (C) in units of μg/L.

$$RF = \frac{\bar{R}}{C}$$

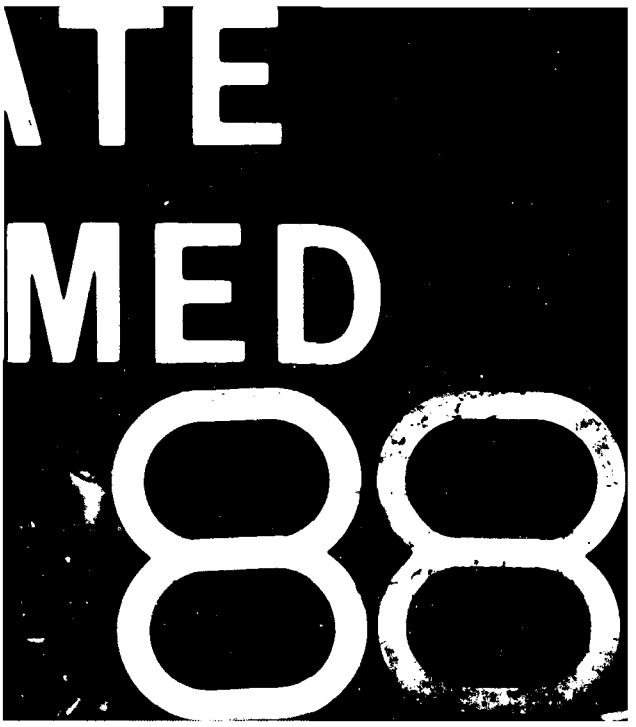
B. ANALYTE CONCENTRATIONS. Solution concentrations (μg/L) in the water samples (C_a) are obtained by dividing the response obtained for each sample (R_a) by the response factor

$$C_a = \frac{R_a}{RF}$$

IX. DAILY QUALITY CONTROL

A. CONTROL SPIKES. Spiked water samples are prepared as described for Class 1 methods in the USATHAMA QA Manual (2nd Edition, March 1987). For each analytical lot, a method blank, a single spike at two times the certified reporting limit and duplicate spikes at ten times the certified reporting limit are analyzed for each analytical lot. Control spikes are prepared using the appropriate spiking solution in a manner identical to that described in section V.

B. CONTROL CHARTS. The control charts required are described for Class 1 methods in USATHAMA QA Manual (2nd Edition, March 1987). Standard Shewhart $\bar{X}$ and R chart for the duplicate high spikes and moving average $\bar{X}$ and R charts for the single low spike are required. Details on the charting procedures are specified in USATHAMA QA Manual (2nd Edition, March 1987).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